RESEARCH ON THE INITIAL FACTORS OF MORPHOGENESIS
IN ANURAN AMPHIBIA.
PART I.   RESULTS OF THE SCHULTZE EXPERIMENT
AND THEIR INTERPRETATION

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With respect to the initiating factors of morphogenesis in
tailless amphibians, the results of the Schultze experiments
(compression and inversion of the ovum after or at the begin-
ning of segmentation) are interpreted and modified, using the
fertilized undivided ova of Rana fusca, Rana esculenta, and
Discoglossus pictus as experimental material. It is demon-
strated that all points on the surface of the ovum are able
to form a dorsal blastopore lip, provided that certain con-
ditions for appearance of the prodromes of morphogenesis are
met, including contact between the white vitelline substance
of the vegetative pole and the pigmented substance. The
prodromes of morphogenesis are due to an interaction of
chemical nature between the vitelline gradient and a cortical
substance distributed over a cortical dorsoventral field
whose intensity decreases progressively from a dorsal focus.
The dorsal lip of the blastopore forms the true nodal point
of a complex system and has a dominant physiological action
on the less developed lateroventral regions. The vitelline
gradient determines the cephalocaudal polarity.

CONTENTS

1. Introduction

The success of experimental studies on the germ of Amphibia during the past 20 years is a well-known fact. Nevertheless, the analysis of actual development has not been pushed with equal rigor in all stages: Until very recently, most researchers have centered their attention on the advanced blastula or gastrula. The results obtained to date make it possible to attribute to the ovum, at the eve of gastrulation, a certain number of properties constituting the essential bases of subsequent morphogenesis; these can be designated, with full justi- /630 fication, "prodromes of morphogenesis".

These prodromes are of three types: 1) the organizer of Spemann (1913), where a cephalic and a truncal zone can be distinguished (Spemann, 1931; Hall, 1932-37); however, this distinction is of a quantitative rather than of a qualitative character (Dalcq, 1936-38). 2) A constellation of tendencies of well-defined morphogenetic movements (epiboly - invagination - extension - convergence) that exhibit a certain autonomy ["dynamic determination" (Vogt, 1922, 1923, 1929); see also Holtfreter, 1933]. The highly particular distribution of the presumptive anlage over the surface of the blastula (Vogt, 1929) is nothing else but an expression of the constellation of these "morphological tendencies". 3) Finally another constellation of local predispositions exists which assuredly are labile but, under certain experimental conditions such as translocation, may exhibit a notable stability (Dalcq, 1936-38; Bautzmann, 1933).

This enumeration can only emphasize the apparent disparity of these essential factors of morphogenesis. The great regulatory capacity, due to the presence of the organizer, is difficult to reconcile with the existence of local predispositions refined to the point of respecting the three nephrons of the pronephros (Dalcq, 1936-38). On the other hand, if the organizer would thus literally control the morphogenesis, it is difficult to detect any link between this center and the tendencies to epiboly of the epiblastic substance and to invagination of the ventral marginal substance, which persist after isolation of these regions. Similarly, it is difficult to conceive the reason why the experimental interventions should so readily dissociate the morphogenetic movements while they are so admirably coordinated in the normal development.

The origin of these prodromes poses just as difficult a problem. To /631 what degree do they pre-exist from the very first stages of the segmented ovum, even of a nonfertilized ovum? Here again, the preformationism - epigenesis dilemma re-appears.

The most preformistic concept consists in attributing, to the nonfertilized ovum, integrality of the prodromes of morphogenesis. Elaborated during ontogenesis, these prodromes would be in a way "latent", awaiting, for initiation, the intervention of an intercurrent factor such as a sufficiently advanced cellularization or the intervention of a nuclear factor. Two different groups of experiments seem to corroborate such a hypothesis: the ligatures effected by Fankhauser (1930) a few minutes after fertilization of the triton ovum and the punctures made by myself (1932), again a few minutes after fertilization but on the frog ovum. In both cases, the results of these premature interventions differed barely from those of similar operations performed at much later stages: The results by Fankhauser can be superimposed on those by Ruud and Spemann

(1922, operations on the gastrula) and my own on those by Suzuki (1928, operations on the blastula). Thus, Fankhauser arrived at the conclusion that "the organizer center is localized, already shortly after fertilization, on the future dorsal end of the ovum" (loc. cit., p.713). A critical study of the experimental results obtained with other animal forms induced the author to make the justified remark: "So far as I can see, it has never been proved by experiment that the first determination processes in the ovum are initiated directly by the fertilization" (loc. cit., p.726). Thus, Fankhauser believes himself entitled to state: "Considered in this connection, the possibility becomes of increased interest that, also in the amphibial ovum, the first and most important determination step might take place already before fertilization" (p.726).

Despite the fact that the deductions by Fankhauser were quite carefully worded, they seem to exceed, in some respect, the range of actually observed /632 facts. The last conclusion, cited here, no doubt is entirely justified. However, can one consider that the existence of an organizer in the dorsal region of the undivided ovum is actually proved? What the ligature experiments have demonstrated is merely the existence, in this dorsal region of the unsegmented ovum, of a certain differentiation which is determining for the bilateral symmetry. There is no doubt that this differentiation topographically coincides with the organizer of the subsequent stages and that the organizer actually forms the chronological extension of the precocious differentiation. However, does this justify, without further substantiation, to assimilate these completely? The concept of "organizer" has been defined only for a certain number of physiological properties; to attribute the function of "organizer" to a unsegmented portion of the ovum means attributing to it also physiological properties, which is by no means certain and not very likely.

As for myself, I had gone farther and even too far. In fact, as done by Fankhauser, I had assumed implicitly the existence of an organizer in the unsegmented ovum. However, I had mentioned at the same time that "a detailed study of the lesions permits the conclusion that an exclusive involvement of the organizer center is not sufficient for explaining the observed deficiencies" (loc. cit., p.571). Considering the reservations made on the possible existence of an organizer in the undivided ovum, this conclusion remains valid. In fact, it is certain that the consequences of punctures made by me can be understood only if the morphogenetic organization of the ovum is not limited to a single restricted zone – for example, to the region of the gray crescent. However, I was definitely wrong in believing that, aside from the organizer, so-called "germinal localizations" or "predetermined territories" were in existence. These concepts, which were much too static, must be rejected today.

In addition, the method of punctures, and specifically those of an undivided ovum, is unthankful: The method neither permits determining nor appreciating the degree of the lesion. To this must be attributed a manifest error, as /633 shown also here. I had concluded that the "morphogenetic territories" must be located in the depth of the ovum rather than on its surface. At that time, I based these conclusions on the fact that the ova, having ejected an extraovate, exhibited deficiencies whereas those that only had shown an eschar remaining enclosed in the cortex, had developed normally; in reality, the lesion in the first case most likely had been much more extensive.

Overall, what this investigation demonstrated was the following: a) There existed a certain differentiation and a certain organization in the ovum of the Anura, a short time after fertilization, without the possibility of further identification; b) this organization did not change, as had been expected, at the instant of appearance of the gray crescent.

In opposition to these hypotheses, Wintrebert (Ref.47) has made an attempt to develop a theory of so-called physiological epigenesis. This effort was quite useful and - in its principle - laudable; no doubt, Wintrebert had the considerable merit of emphasizing the major role played by epigenesis in the development. However, his attitude was much too radical and, a much more serious matter, the epigenetic mechanisms described or imagined by this author are far removed from reality (see Dalcq, 1936; Pasteels, 1936-38).

In my previous paper on the gastrulation of Discoglossus (1936), I formulated the following conclusion: "There is no doubt that the organizer pre-exists - in an inactive form which the future will have to define - in the blastula, in the morula, and even in the undivided ovum". The reservation "in inactive form" seems inadequate today. Nevertheless, I insist on the fact that this changes nothing in the scope of refuting Wintrebert's arguments. If, in principle, this author would have been less wrong than I believed then, his interpretation would actually have been based on gross errors.

The results obtained by Penners and Schleip (1928) in a repetition of /634 the Schultze experiment (compression and inversion of the ovum after or at the beginning of segmentation) should have drawn attention to the fact that the morphogenetic organization of the undivided ovum must in reality be quite different from that of the blastula. Whereas it is known that, in the blastula or in the gastrula, an embryonic axis can be obtained only in close correlation with the organizer, Penners and Schleip have demonstrated in an irrefutable manner that all points on the surface of the ovum are able to form a dorsal lip of the blastopore, provided that certain conditions are satisfied. An essential factor in this respect consists in a contact between the white vitelline substance of the vegetative pole and the pigmented substance. Nevertheless, the zone of the gray crescent - previously defined - of the normal development remained distinctly preferred: In the great majority of cases, the blastoporal lip had a distinct tendency to manifest itself along the margin of a vitelline conglomerate, but closest to the gray crescent. Penners and Schleip concluded with justification that, if any differences existed in the surface layer of the ovum with respect to the capacity of forming a blastoporal lip, they could be only of a quantitative order. In fact, the interpretation by these two authors starts presenting difficulties only when it becomes a question of defining the action of the "gray crescent". Primarily, the authors considered three possibilities ("the localization of the primitive blastopore anlage is determined by the special capabilities of the region of the gray crescent or by the influence of the white substance on adjacent darker substances or by both moments together", 1928a, p.404) without, however, deciding in favor of one of the three. The resultant element of uncertainty turns into a true perplexity when considering that the authors start confusing the gray crescent of the unsegmented ovum with the organizer of the gastrula. From then on, it is virtually impossible /635 to understand their reasoning. Penners and Schleip insist on the fact that the active substance, located at the level of the gray crescent, compared to the

organizatory center, is considerably stable and is not displaced by the altera-
tion of the vitellus; however, they insist at the same time on the fact - as
recently and irrefutably demonstrated by Penners (1936) - that a blastoporal
lip, in certain cases, may form in the ventral half of the ovum. Presented in
this manner, the facts appear contradictory and are in complete opposition to
all other experimental data collected on Amphibia. The consequences are rather
unfortunate. In 1929, Penners concluded as follows: "Thus, the concept that
the organizer in the gray crescent of the frog ovum is not absolutely essential
for the formation of an embryo seems more acceptable than the opposite opinion.
In fact, independent of the normal effective organizer an additional center
(possibly even several) may be formed" (loc. cit., p.101), which removes all
explanatory value from his otherwise elegant experiments. As a consequence,
the excellent works of the authors, instead of arousing the interest that they
would very much deserve, are looked upon more or less as curiosities difficult
to integrate into a general frame. To prove the point, it is sufficient to con-
sult recent monographs: Dalcq (1935) as well as Spemann (1936) appear rather
troubled by the results of Penners and Schleip and suggest that these could pos-
sibly be explained by a dislocation of the organizer, a version that, however,
by no means agrees with the data of these authors.

At the bottom of all these difficulties is the error common to the great
majority of embryologists: the necessary assimilation of the dorsal differentia-
tion of the undivided ovum (gray crescent) and of the organizer of the blastula.
In the case of Penners and Schleip, this error is the more surprising as their
results (as demonstrated below) indicate that the organizer, during the seg- /636
mentation, is built up from a more elementary structure.

However, a new stage had to be traversed before arriving at this decisive
conclusion. This comprises the valuable experiments by Vintemberger which
furnish the elementary bases. The colored tracings made by Votquenne (1934)
for Rana fusca demonstrated that, in this species, the micromeres of the stage
VII comprise the entire center of the notochord and thus a large portion of the
territories that topographically correspond to the organizer. Nevertheless, the
isolation of these micromeres achieved by Vintemberger (1935) did not result in
morphogenesis. The following year, the Strasbourgian author repeated these
experiments, combining them this time with a transplantation, on a base, of
vitelline cells taken from the vegetative pole of a blastula. The result is
obvious. The isolated micromeres differ from the chordomesoblast only in excep-
tional cases. When implanted on a base of vitelline cells, the micromeres, in
the majority of cases, will build up axial organs. So far as the conclusion by
the author is concerned, we are giving it here in its entirety: "This result
seems to indicate that the development, in connection with the mass of vitelline
cells, is one of the factors necessary for realization of the potentialities of
the organizer in the normal ontogenesis" (1936, p.930).

Certain observations by Motomura (1935) also emphasize the role of the
vitellus in morphogenesis. This author repeated the Schultze experiment but
used a Urodela, Hynobius lichenatus. It seems that the development of this
species is very slow, in fact much slower than that of anurans. Presumably,
this is responsible for the fact that, in Hynobius, the inversion of the unseg-
mented ovum produces a total reversal of polarity, with the animal pole becoming
filled with vitellus and the vegetative pole becoming drained of it. (In Rana,

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as noted by Penners and Schleip and as demonstrated below, a vitelline con-
glomerate still persists at the level of the primitive vegetative surface; in
only a few cases will the vitellus be able to traverse the ovum from end to end
and produce a vitelline conglomerate at the primitive animal pole.) Another
discrepancy is more difficult to explain: In contrast to what happens in the
frog, the starting point of invagination (50° under the equator of the normal
ovum) remains especially stable in Hynobius. The blastoporal lip of the in-
versed ova forms at the normal site but the sense of invagination is diametrical-
ly opposed, which means that, in the normal ovum as well as in the inversed
ovum, the invagination always follows the same gradient, starting from the most
vitelline point and proceeding toward the less vitelline regions. Motomura was
entirely justified in concluding from this that the craniocaudal axis of the
embryo is determined by the composition of the ovular cytoplasm, i.e., by the
vitelline gradient. Let us add here that, if it is true that the starting point
of the blastoporal lip has not varied it must also be true that the organizer
must have formed between this point and the equator in the normal ovum and be-
tween this point and the primitive vegetative pole, in the inversed ovum.

This even suggests the idea that one of the prodromes of morphogenesis,
namely the organizer – which most likely also is true for the others – is the
result of an epigenetic evolution in which the vitellus plays a predominant role.
It is also necessary to know from which elements this epigenesis originates. As
rightly mentioned by Dalcq (1935, p.43): "A minimum of preformation is logical-
ly and objectively ineluctable and the epigenesis – no matter how vast its
domain – will never be anything but relative".

Along this same reasoning on "the regulation in the germ and its interpreta-
tion", Dalcq arrived at the conclusion that "the necessary and sufficient factors
for morphogenesis" are of three orders a) an axial gradient, b) a specialized
substance, c) a complete genoneme. The necessity of nuclear integrity is demon-
strated in investigations by Dalcq (1931) and by Dalcq and Simon (1932) (intoxi-
cation of the spermatic nucleus by trypaflavine and radiations, cauteriza-
tion of the maturation macula) as well as by the hybridization experiments in
combination with merogony, performed by the Baltzer school. In connection
with the "axial gradient", studies by Penners and Schleip, by Vintemberger, and
by Motomura have drawn attention to the vitelline polarity. So far as the
"specialized substance" is concerned, this obviously involves an enigmatic
factor which is localized on the dorsal end of the ovum (gray crescent) and de-
termines the bilateral symmetry.

At present, one can state that a predecessor element of the "dorsal sub-
stance" pre-exists in the nonfertilized ovum. A generalization of certain
aspects of fertilization in Rana fusca seemed to indicate that the penetration
of the spermatozoid determined the bilateral symmetry plane in amphibians
(W.Roux) or at least modified it (A.Brachet). In reality, the facts are much
more complex. In the case of Discoglossus pictus the supposed action of the
sperm-aster on the bilateral symmetry (Wintrebert) is based on errors of ob-
servation; the strictly axial fertilization thus can have no effect on the
localization of the gray crescent (Pasteels, 1938a). In Rana fusca (Ancel and
Vintemberger, 1938), the entering point of the spermatozoid determines the bi-
lateral symmetry only if the ovum is in a vertical position; if the ovum is in-
clined, the fertilization meridian is pre-established, and the direction of

rotation of the ovum in its perivitelline determines the dorsal side. In Rana esculenta (Pasteels, 1938a and b), two factors at least are active: a) the eccentric position of the germinal vesicle in the oocyte; b) as in Rana fusca, the mode of rotation of the ovum in its perivitelline (see also Dalcq and Pasteels, 1938).

Seen from an overall viewpoint, fertilization only sets in motion certain wheelwork pre-existing in the nonfertilized ovum. The exact nature of these mechanisms and the slow and progressive work of epigenesis that they initiate are problems that should be settled at present.

Since the Schultze experiment had been so fruitful in its modification   /639 by Penners and Schleip whereas the interpretation by these authors still left some obscure points, it seemed of interest to resume these experiments, at least in a first stage. In all cases in which rotations had been imparted to the undivided ovum during the first segmentation or even during the stage II, emphasis was placed specifically on the position of the gray crescent which was carefully outlined on each ovum before starting any experiment.

As material, we used ova of Rana fusca, Rana esculenta, and Discoglossus pictus; the ova of the first species were harvested in the conventional manner, after opening highly gravid females. Of the green frog it is known that this species rarely matures in captivity. To avoid this drawback, the females were injected daily with a ground mass of three frog hypophyses in physiological serum (data by Gallien, 1937). As soon as the ova had descended, the females were sacrificed and the ova were fertilized with sperm obtained from ground testicles. This made it possible to obtain spawns from the end of April to June 15.

As method, we literally followed the technique given by Penners and Schleip: compression of well-aligned ova between two slides joined at their extremities by two small plastiline blobs. The gray crescent or its equivalent in Rana esculenta was outlined with a dermographic crayon on the upper of the two slides. The compression must be sufficiently strong to keep the ovum properly fixed. Except in Discoglossus, one usually reaches the limit of cortical resistance so that these operations leave considerable waste.

Examination of the living germ is followed by fixation in Bouin's fluid and staining with hematoxylin-eosin.

To some extent, the obtained results exceeded the expectations: Numerous obscure points became quite clear and, specifically, many of the disparate data became coherent. During animated discussions in June 1937, Prof. Dalcq and the author compared the conclusions of research done by both. In this manner, we formulated a general interpretation, qualified as "new concept of physiological bases of morphogenesis" (Dalcq and Pasteels, 1937, 1938). The present paper /640 constitutes a substantiation of the first part of the theoretical aspects expressed in the above-mentioned articles, to which the reader is referred for further details.

2. Results

Counting only ova whose gastrulation and first morphogenesis stages had actually been observed, we had the following material available:

Rana fusca	95	embryos
Rana esculenta	29	embryos
Discoglossus pictus	10	embryos
	Total 134	embryos.

All these embryos were most carefully followed up and delineated at least three times during the gastrulation. Each time, they were removed from the compressor as soon as the first blastoporal lip appeared (ova left in the compressor, because of the mechanical gene showed a peculiar evolution which will be described in a future paper). In all these ova, the gray crescent was located in a reliable manner.

On the whole, the obtained results agree with those by Penners and Schleip: appearance of blastoporal lips at the margin of white vitelline cell conglomerates; more or less close connection of these lips with the previously spotted region of the gray crescent; formation of multiple embryos; possibility of morphogenesis initiation from all points of the ovum; etc. Thus, there is no need to give further details here. For the remainder, the reader is referred to the papers by Penners and Schleip; we will give here only a few typical examples for the interaction of the vitelline conglomerates and the region of the gray crescent, in so far as initiation of morphogenesis and most logical /641 interpretation are concerned.

a) Rana fusca

As already described by Penners and Schleip, total inversion of the ovum of R. fusca, kept in the Schultze compressor, rapidly initiates a flow of vitelline substances toward the animal pole* which now has shifted to the bottom; this flow can take place either along the groove of the first or second segmentation or directly into the depth of the ovum or else across the entire substance, thus reaching the surface layers of the animal pole. The heavy vitellus, at the instant at which it reaches the cortex of the ovum, forces the subcortical pigment backward; this results in a large vitelline conglomeration at the level of the cortex, having the white aspect of the vegetative pole of a normal ovum. A moderate accumulation of vitellus at the level of the cortex would show the gray aspect so characteristic of the marginal zone.

However, the modification of the ovum is relatively slow so that it will be literally congealed by the cellularization well before the process is complete. As a general rule, a white vitelline conglomerate, of a size varying in accordance with the individual case, will remain at the level of the vegetative pole;

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* These terms "vegetative and animal pole" naturally always mean the primary vegetative and animal poles of the normal ovum; similarly, the "dorsal" end will always be that corresponding to the gray crescent.

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in certain cases, only a gray streak remains, extending toward the equator and even going beyond it. The formation of a white vitellus conglomerate at the animal pole is quite rare. In this case, incidentally, there will always be two conglomerates, one at the vegetative pole and the other at the animal pole.

Below, we will present a few individual types. In all illustrations pertaining to these, the center of the gray crescent – previously delineated – /642 is marked by an asterisk. Naturally, its highly particular pigmentation has been obliterated by the vitelline modification. The markings p.a. and p.v. mean that the ovum is viewed respectively from its primitive animal pole or from its primitive vegetative pole.

Embryo D 1 (Fig.1). – On April 1, 1937, the ovum has inverted at the instant of appearance of the first segmentation groove. Thirty minutes later, the division is completed and (Fig.1a) the vitellus has descended in a streak along this groove, toward the dorsal end. The next day, the blastula (Fig.1b) shows a gray streak starting from the vegetative pole (Fig.1b, p.v.), crossing the equator on the dorsal end, and stopping at midheight of the animal half of the ovum (Fig.1b, p.a.). A few hours later (Fig.1c), a blastoporal groove, oriented along the
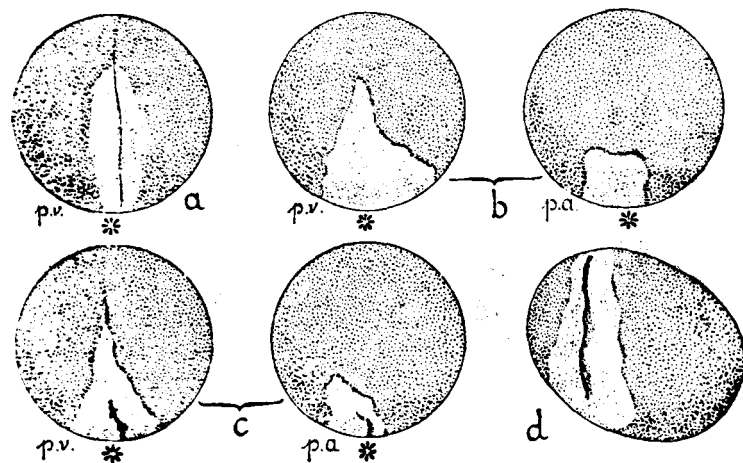

Fig.1 Rana fusca; Inversion of an Ovum at Stage II, Constitution
of a Vitelline Streak along the First Segmentation Groove,
Formation of Two Blastoporal Lips Osculating at the Point
of Contact of the Gray Streak and the Center of the
"Gray Crescent".
p.v. = vegetative pole; p.a. = animal pole; * = center
of the "gray crescent".

major axis of the streak, is outlined at the equator of the ovum, extending toward the vegetative pole and toward the animal pole. These are two blastoporal lips juxtaposed on the right and left and each invaginating separately (see Penners and Schleip, 1928, p.427 : "gastrulation without formation of a vitelline plug"). At the end of the second day (Fig.1d), the blastoporal groove has spread considerably. On April 3rd, formation of a duplicitas anterior was

observed.

Summary. – Absence of a white vitelline conglomerate; "marginal" streak /643 crossing the equator in the dorsal region; appearance of two juxtaposed blastoporal lips, exactly at the level of the center of the gray crescent; extension of the blastopore along the major axis of the vitelline streak.

Sixteen other similar cases yielded the same results. The gray streak, depending on the variable position of the first segmentation plane, did not always reach the equator at the level of the dorsal region; the first blastoporal lips appeared at the point closest to the gray crescent.

Embryo C 3 (Fig.2). – On April 1, 1937 at 10 AM there is an inversion of the undivided ovum, but already carrying its gray crescent. Twenty-four hours later (Fig.2a), the ovum shows a large irregular gray streak at its center, and a small vitelline conglomerate slightly to the left of the vegetative pole; a blastoporal lip appears on the dorsal end of this vitelline plug. Two hours later, a second blastoporal lip appears facing the first one, i.e., on the ventral side. The two lips join at the level of the vegetative pole (Fig.2b). On the evening of this second day, the gastrulation has progressed further /644 and the two blastoporal lips have joined in form of a spindle, enclosing a vitelline plug. On the third day, a duplicitas lateralis can be discerned.
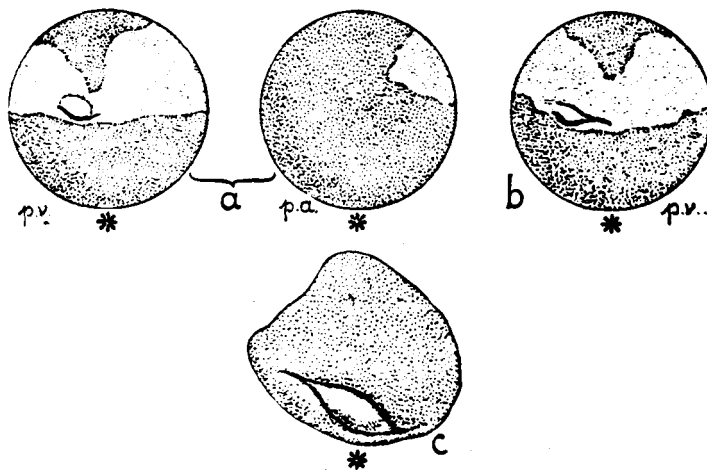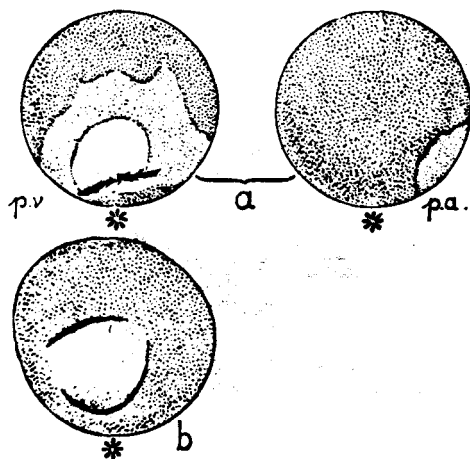

Fig.2 Rana fusca; Formation of Two Blastoporal Lips around a
Small Vitelline Conglomerate; the Blastopore Appears
First on the Dorsal End.
p.v. = vegetative pole; p.a. = animal pole; * = center
of the "gray crescent".

Summary. – Small vitelline plug at the level of the vegetative pole; two blastoporal lips on either side of this conglomerate; first appearance of the blastopore at the dorsal end; ventral blastopore appearing a short time after the first.

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Six other embryos underwent a similar evolution. Obviously, the position of the vitelline conglomerate is variable. However, in all cases the first blastoporal lip formed on the dorsal end. Similarly, in each case the chronological interval between the two lips was not very great and did not exceed 2 - 3 hrs (at the same temperature, gastrulation takes about 15 hrs to be completed).

Embryo E 6 (Fig.3). - On April 2nd at 3 PM, the ovum has inverted at the stage II, while the second segmentation groove is already outlined. On the next day, at 10 AM (Fig.3a), a large gray plug is observed at the center, and a relatively large white vitelline mass in the vegetative half of the ovum, /645 extending from the vegetative pole to almost the center of the gray crescent. With respect to this point, a large transverse blastoporal groove underlines the entire dorsal margin of the vitelline conglomerate. Eight hours later (Fig.3b), a new blastoporal lip is outlined on the ventral side of the vitelline conglomerate, facing the first one which now has assumed a crescent shape. On the third day, there is formation of a duplicitas ventralis which, incidentally, is undergoing necrosis.

Fig.3 Rana fusca (see Fig.2); Larger Vitelline Conglomerate; Greater Chronological Interval between the Two Blastoporal Lips.
p.v. = vegetative pole; p.a. = = animal pole; * = center of the "gray crescent".

Summary. - Case analogous to the preceding; larger vitelline conglomerate; larger space between the two blastoporal lips, increasing the chronological interval between appearance of the two blastoporal lips.

Twelve embryos showed an evolution either analogous to or intermediary between this and the preceding case.

Embryo K 2 (Fig.4). - On April 8, 1937 at 3 PM, inversion of the ovum, segmented in two, takes place. The next morning (Fig.4a) an elongated mass of white vitellus appears at the vegetative pole (Fig.4a, p.v.), two-thirds circumscribed by a marginal zone. On the dorsal end, a blastoporal lip forms at the limit of the white vitellus. Conversely, at the animal pole (Fig.4a, p.a.), a second /646 relatively large and somewhat rounded vitelline conglomerate appears, whose dorsal end is also underlined by a blastoporal lip. Three and one-half hours later, the two vegetative and animal blastopores have progressed farther; specifically the latter is quite remarkable, extending in the form of a Y whose two upper branches circumscribe the vitelline conglomerate while the lower branch extends toward the center of the gray crescent. On the evening of the second day, as shown in Fig.4c (p.v. and p.a.), the two blastopores are just about to close. On the third day, two medullary plaques appear that osculate at their head and soon form a duplicitas cruciata.
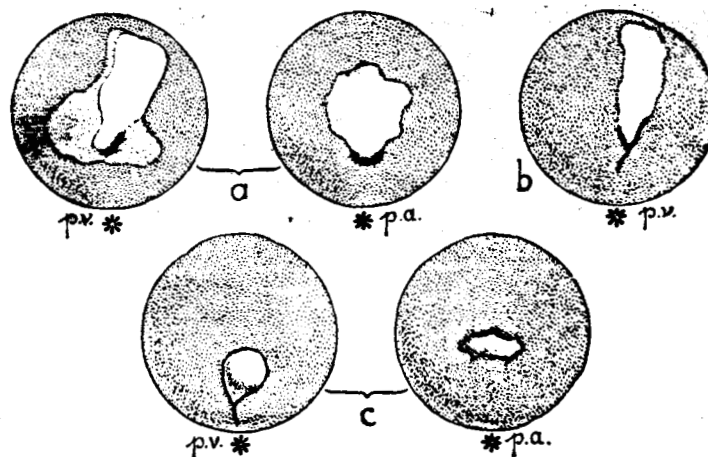
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Fig.4 Rana fusca; Formation of Two Vitelline Conglomerates,
One at the Vegetative Pole and the Other at the Animal Pole;
Gastrulation at the Level of each of these Conglomerates.
Note the deviation of the vegetative blastopore
toward the center of the "gray crescent".

Summary. – Two vitelline conglomerates, one at the vegetative and the other
at the animal pole, each causing – with respect to the center of the gray
crescent – the formation of a dorsal lip of the blastopore. These two vitelline
conglomerates are quite extensive, with a single blastoporal lip forming at the
level of each.

Four other embryos showed two vitelline conglomerates at each of the poles
of the ovum. For all of these embryos, a gastrulation starts at the level of
the two vitelline conglomerates. When these conglomerates are very small,
several blastoporal lips may form at the level of each.

An excellent example is given by Rana esculenta.

Twenty-six other embryos showed a Y-shaped blastoporal lip, starting at the
margin of the vitellus and attracted also toward the center of the gray crescent.

Among these typical cases, described above, numerous intermediate types
exist which need not be discussed further.

b) Discoglossus pictus

The ten embryos of Discoglossus, available to us, more or less are covered
by the above–described cases for Rana fusca; therefore, no further discussion
will be given here.

The inverted ova of the green frog generally show the same behavior as those of the brown frog, the only difference between the two species having to do with distribution of the pigment. It is known that the ova of R. esculenta are much less pigmented than those of R. fusca. The pigmentary cap, of a light mahogany color, hardly projects beyond the equator on the ventral side. On the dorsal end, the equivalent of the "gray crescent" of Rana fusca presents itself in the form of a completely depigmented zone which, in the unsegmented ovum, extends distinctly to above the equator.

A peculiar phenomenon is the fact that this light pigment adheres much more tightly to the cortex than the abundant pigmentation of R. fusca. We have demonstrated that the pigment of the latter species is completely pushed back by the influx of vitellus, which is not at all the case for R. esculenta. Here, after inversion of the ovum, the possible contact of the vitellus with the level of the animal portion of the ovum can be detected only by the volume of the cells: A blastoporal lip could appear at the level of large cells that had re- mained dark. Similarly, at the level of the vegetative pole, the vitelline plugs which are smaller than usual can be differentiated from the ambient marginal zone only by the size of the cells, since the white coloration is identical. Thus, these vitelline plugs cannot be delimited except after a care- ful examination and then only in first approximation. In some respects, the observation is thus more difficult in R. esculenta.

The reason for discussing these details, which in themselves are not of great intrinsic interest, lies in the opportunity they offer for demonstrating a point. Penners and Schleip who, with respect to Anura, experimented only with Rana fusca greatly emphasize the fact that the blastoporal lip appears at the junction of white and brown substance ("white and brown vitellus"); for example, Penners and Schleip (1928a, p.445) stated: "We conclude from this that the de- velopment of darkish or not entirely white substances is initiated by an   /648 influence exerted by the pure-white substance on the darker substance". It is entirely obvious that these authors have the role of the vitellus in mind here. Nevertheless, a certain ambiguity remains: Would some degree of pigmentation not play a certain role in the determination of morphogenesis? The evolution of Rana esculenta ova shows definitely that this is not the case and that only the vitelline density is of importance. The pigmentary modifications of Rana fusca have merely the significance of an epiphenomenon.

On the whole, the cases observed on green frogs show the greatest similari- ties with the above-described types. Thus, no further discussion of this point will be given here and merely a few examples on some new aspects will be pre- sented.

<u>Embryo A 16</u> (Fig.5). – On May 10, 1937 at 6 PM, the ovum has inverted (stage IV). Twenty-four hours later, a relatively large plaque of coarse cells appears at the vegetative pole (Fig.5a). Three hours later, a blastoporal lip appears, facing the center of the gray crescent (Fig.5b). On the third day, the blastoporal groove continues; this groove is unitary but asymmetric and more de- veloped toward the left side (Fig.5c). The resultant embryo is unitary but more developed on the left than on the right.
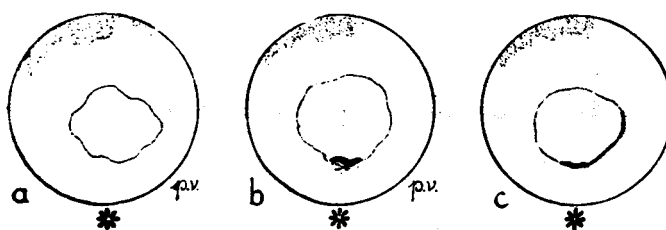
Fig.5 Rana esculenta; Unitary Gastrulation at the Level
of a Large Vitelline Conglomerate.
p.v. = vegetative pole; * = center of the "gray crescent".

Summary. - Large vitelline conglomerate whose size is close to normal. The
blastopore starts at the usual site and, although asymmetric, remains simple.

Two other embryos developed in a similar manner. Numerous examples are   /649
contained in a later paper, treating partial inversions (135°).

Embryo D 4 (Fig.6). - On May 17, 1937, one undivided ovum is inverted by
180°. Twenty-four hours later, two masses of vitelline cells are observed on
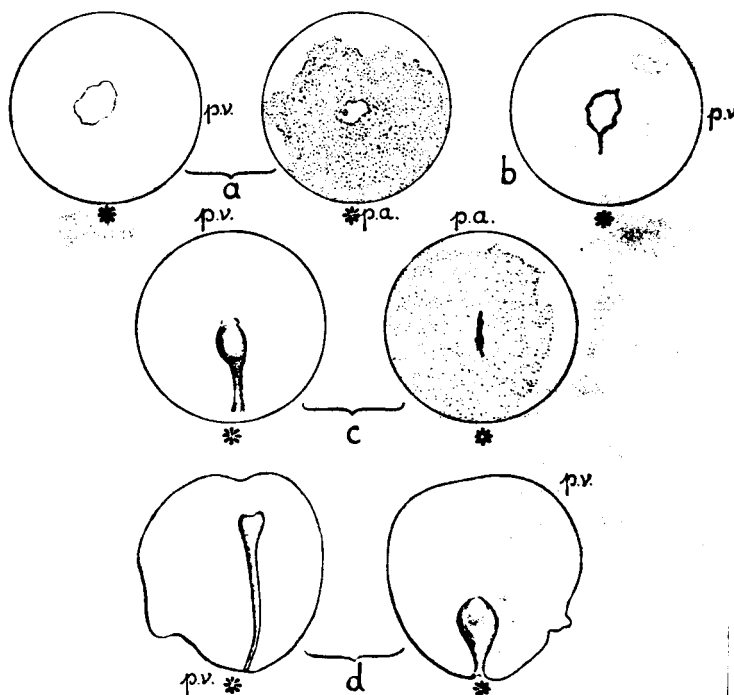

Fig.6 Rana esculenta; Two Vitelline Conglomerates, One at the
Vegetative Pole and the Other at the Animal Pole; Double
Gastrulation around each.
p.a. = animal pole; p.v. = vegetative pole; * = center
of the "gray crescent".

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the blastula, one at the vegetative pole (Fig.6a, p.v.) and the other at the animal pole (Fig.6a, p.a.). These two conglomerates are smaller, particularly that of the animal pole. A few hours later, gastrulation sets in; first, encircling the vegetative cells, two blastoporal lips form that face each other and join in a groove extending toward the gray crescent (Fig.6b). Another few hours later, during which time the vegetative lips have progressed (Fig.6c, p.v.) to the animal pole, a small blastoporal groove is observed outlined at the site of the small vitelline conglomerate (Fig.6c, p.a.). Finally, the two   /650 blastopores join (Fig.6d) and end in a duplicitas ventralis which is poorly conformed and does not exceed the neurula stage.

Summary. - Two small vitelline conglomerates, at each of the poles of the ovum, each initiate the formation of two blastoporal grooves; the latter extend toward the center of the gray crescent and join there.

Eight other embryos showed a similar evolution; in most cases, the blastopores of the animal pole and of the vegetative pole conflict and lead to a rapid abortion of the morphogenesis.

Embryo F1 (Fig.7). - On May 19, 1937, one undivided ovum is inverted by 180°. Twenty-one hours later, a vitelline conglomerate of moderate size persists at the level of the vegetative pole (Fig.7a); the dorsal blastoporal lip is outlined, facing the gray crescent. Eight hours later, when the vitelline conglomerate is almost completely imbedded by the blastopore, it is found that the latter extends along a deep groove which has two lips pointing toward the center of the gray crescent (Fig.7b). At the instant of formation of the ventral lip, the small vitelline plug is surrounded by a tripod blastopore whose main leg is dorsal (Fig.7c). On the third day, a duplicitas anterior appears.
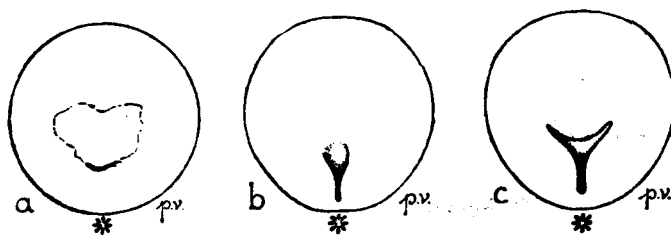
Fig.7   Rana esculenta; Blastoporal Lip Deformed toward
the Center of the "Gray Crescent".
p.v. = vegetative pole; * = center of the "gray crescent".

Summary. - Appearance of the dorsal lip of the blastopore, at the margin of a vitelline conglomerate rather far from the center of the gray crescent, but facing it. During the subsequent development, deviation of this blastoporal   /651 lip toward the gray crescent.

Six other embryos demonstrated a similar attraction of the blastoporal lip toward the center of the gray crescent.

3. Interpretation

The above-described facts confirm a certain number of conclusions drawn by Penners and Schleip: a) There is no doubt that an embryonic axis may originate at no matter what point of the surface of a given ovum; b) in Rana fusca, the starting point of the blastopore always is in contact with a conglomerate of white vitelline cells and pigmented substances; c) the location of the dorsal lip of the blastopore presents very intimate connections with the region of the gray crescent. Everything proceeds as though there existed at the surface of the ovum, with a maximum at the level of the gray crescent, quantitative differences from a factor predisposing the formation of the blastopore.

In certain respects, we were able to obtain closer and newer definitions: a) If a blastopore is formed at the junction of a white vitellus conglomerate with a strongly pigmented region, as is the case in Rana fusca, the decisive factor here will be a certain concentration of vitellus; the accumulation of pigment in vitellus-poor cells is due only to an intercurrent cause and has no effect on the final result. b) Penners and Schleip (1928a) noted that "the localization of the blastopore is determined either by the special properties of the region of the gray crescent, or by the influence of the white substance on the dark substance contiguous to it, or else by a combination of both factors" (cf. 1928a, p.404). The totality of our observations permits the statement that the localization of the first blastoporal lip definitely is due to the combination of a certain vitellus accumulation with the "gray crescent" factor. There is no indication that this localization may result from one of these /652 factors alone. Conversely, all above-analyzed examples can be readily explained as being the result of the necessary combination of a vitelline factor with some other factor which has its concentration maximum at the level of the gray cresent.

a) The dorsal lip of the blastopore and the lateroventral lips following the former actually appear always at the base of a vitelline gradient. In fact, starting from the margin of a vitelline conglomerate, a veritable decrement in vitellus exists which manifests itself in the peculiar aspect of "marginal" gray cells that surround the white conglomerate and gradually fade into the brown substance of Rana fusca. Thus, this vitelline gradient has its maximum at the level of the large white cells and gradually diminishes in a centrifugal direction. This not only has the effect of localizing the blastoporal lip but also to control the sense of invagination. As in the experiments by Motomura (1935), the vitelline gradient determines the orientation of the cephalocaudal axis of the organism.

Two possibilities are in question: 1) As a consequence of the inversion of the ovum, a streak of gray cells without conglomerate of coarse vitelline cells is formed. On either side of this streak (see Fig.8c), 2 gradients radiate in opposite directions: Two blastoporal lips originate on contact with each other, at the level of the common base of these two gradients, and then invaginate in opposite directions in accordance with the two gradients. This constitutes a "gastrulation without formation of a vitelline plug", as described by Penners and Schleip. 2) Either at the vegetative pole or at both poles of the ovum, a conglomerate of coarse white cells exists. The inordinate accumulation of vitellus, presumably due to an excessive dilution of the cytoplasm, apparently

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produces a sort of inertia: The blastoporal lip appears at the external limit of the white mass; this mass which later will constitute the "vitelline plug" is passively entrained to below the blastoporal lip and there constitutes the /653 entoblastic mass of the archenteric floor. A vitelline gradient of centrifugal direction will spread all around the conglomerate of white cells (Figs.8a and b).
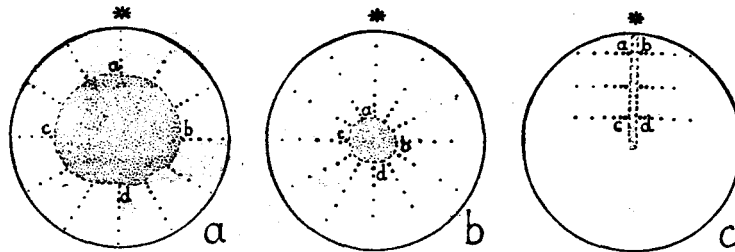

Fig.8 Schematic Layout of the Vitelline Gradient in
Various Experimental Possibilities (See Text).

Thus, the polar gradient of the amphibian ovum, i.e., the "axial gradient" which Dalcq (1935) considered as one of the necessary and sufficient factors for morphogenesis, can be compared to the gradient constituted by the more or less extensive accumulation of vitellus in the various territories of the ovum.

b) The localization of the dorsal lip of the blastopore depends on still another factor: The dorsal lip naturally is always located at the base of the vitelline gradient but, again in all cases, also at the point closest to the center of the gray crescent.

Two remarks should be made in this respect. The first concerns the position of the gray crescent itself with respect to the ovular axis. In Rana fusca (with minor variations, this is true also for the other Anura), the gray crescent is located at the level of the equator of the undivided ovum (see Pasteels, 1932; Votquenne, 1934; Vintemberger, 1933-34). During the segmentation, subsequent to the blastocele cavity, the region of the crescent is shifted into the lower hemisphere of the ovum (Vintemberger, 1934). For inversed ova, in which the blastocele (or blastoceles) is most irregular, both with respect to size and site (see Schleip and Penners, 1928a), the evolution obviously will vary from one case to the other, and the center of the gray crescent will be either /654 slightly above or – most often – slightly below the equator of the blastula. In practical application, this center could be fixed – although slightly arbitrarily – to its primitive position at the level of the equator.

Second remark: Caution must be exerted in considering the "crescent" as being gray in the strict sense. This depigmented zone results from a shift of the superficial substances which, at the instant of conjugation of the pronuclei, undergo a movement of convergence and ascension from the dorsal end of the ovum (see Ancel and Vintemberger, 1933-34). The crescent shape results from the fact that the ascending motion of the pigment decreases progressively as one moves away from the median line. Despite the fact that the gray crescent represents an excellent reference point, at least in Anura, for detecting the dorsal end

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of the ovum, there is no reason to attribute special properties to the entire zone defined (and at that, rather poorly) by this crescent. This is an error occasionally committed by Penners and Schleip and especially by Weigmann (1927) who attribute a particular value to the entire crescent, including its horns. The abundant data available to us permit stating that, around the central region of the crescent above its convexity as well as below its concavity and in the horns themselves, points are encountered which – at equal distance of this central zone – have exactly the same properties.

In fact, the blastoporal lip always appears opposite the center of the gray crescent, no matter whether the vitelline conglomerate is located at the level of the animal pole, of the vegetative pole, or in a lateral region. Naturally, if a conglomerate or vitelline streak comes into contact with the central point of the crescent, the gastrulation will start at this more or less "focal" point. If a small vitelline conglomerate is located at some distance from this focus, the first blastoporal lip will appear facing it; several hours later, a second lip will appear at the opposite vitelline margin. Here again, this evolution can take place indifferently at the animal pole, at the vegetative pole, or laterally. Thus, at the level of the dorsal end there exists an optimum /655 condition which determines the appearance of gastrulation; this condition decreases progressively in the same manner in all directions, toward the ventral side.

This decisive factor of morphogenesis is thus distributed over territories of the ovum where it can play only a subordinate role in the normal development. In fact, at the level of the animal pole there exists a factor capable of starting a gastrulation, provided that a vitelline conglomerate had been carried to there. In extent, this factor greatly exceeds the useful zone. Here, a field in the sense stipulated by P.Weiss exists, known as the dorsoventral field.

The structure of this field is remarkably rigid. The descent of the vitellus (varying in accordance with the individual case) across the substance of the ovum as a consequence of its inversion must necessarily alter the arrangement of numerous substances (backup of the subcortical pigment in Rana fusca). The dorsoventral field never seems affected by this. To what might this rigidity be due? To a microstructure of the cytoplasm which would not be affected by displacements of coarse substances? The manner in which the vitellus is able to flow freely through the ovular substance and the present-day knowledge on the viscosity of the cytoplasm (see Heilbrunn, and others) renders this hypothesis quite unlikely, at least so far as the interior of the ovum is concerned. This leaves the cortex to be considered, and even its topmost layer outside of the pigmentary layer. With respect to the cortex, its structural rigidity would not be a surprising property. On the other hand, the role of the cortex in the morphogenesis apparently is rather large, since the factor initiating the appearance of a blastoporal lip is the actual contact of a vitelline conglomerate with the ovular surface, irrespective of the possible distribution of the vitellus in depth.

Consequently, the dorsoventral field should be considered as being distributed over the surface, thus making it a cortical dorsoventral field. However, one objection can be raised here: Might the totality of the results not be /656 amenable to some other explanation? Instead of a cortical and rigid structure

could one not assume that the factor "gray crescent" is a particular quality normally linked to the vitelline margin? Even if, by inversion of the ovum, the vitelline mass had been dislocated and modified, one could still understand that, in this case, the blastopore appears preferentially facing the side which had been the dorsal end. This eventuality can be reconciled with some results but is distinctly excluded by others. How could one otherwise explain the fact that, in the examples of Figs.2 and 3 (embryos Rana fusca, C 3 and E 6, see pp.10-11), a second blastoporal lip appears on the ventral side with a certain lag whenever the vitelline conglomerate is small. In the hypothesis of the dorsoventral field, this appearance is perfectly logical since, if the vitelline conglomerate is small, the two dorsal and ventral extremities of the vitelline conglomerate will not be far apart so that the quantitative differences of the field will not be excessive.
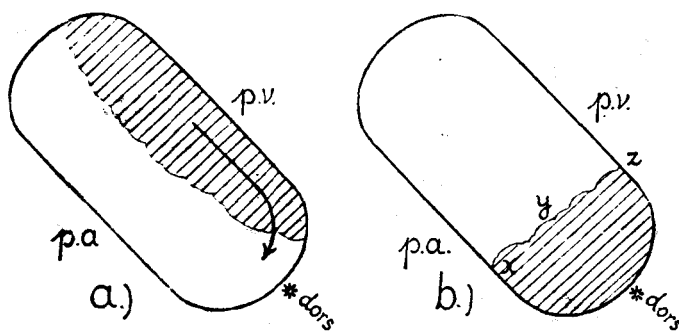

Fig.9   Schematic Sketch for the Possibilities of
Localization of the Dorsal Blastopore Lip after
Rotation through 135°, with the Dorsal End at
the Bottom (See Text).

Still more convincing are the experiments in which the entire vitelline mass is made to slip along the region of the gray crescent. Let us assume that we are inversing an ovum by 135° instead of 180°, with the dorsal end thus moving to the bottom (Fig.9a). The vitelline mass, instead of partially penetrating into the interior of the ovum, will slip along the cortex and occupy the entire dorsal zone of the ovum (see Fig.9a and b). If the dorsal factor would be /657 more or less mobile and linked to the vitelline margin, it would be pushed back by the descent of the vitellus toward the animal pole. In Fig.9b, the blasto- pore necessarily had to appear at the point x. Conversely, if a rigid field truly exists, having its maximum at the level of the dorsal zone, then the blastopore could indifferently appear at the points x, y, and z located at almost the same distances from the focal point of the field, no matter whether they might be beyond the crescent, on this side of the crescent, or laterally located.

This experiment was made on 21 embryos[*]; the results are given in the accompanying table.

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[*] These embryos will be studied in more detail in another paper of this series.

Species	Case	Dorsal Blastopore Lip	Anim. H(x)	Veget. H(z)	Lat. H(y)
Discoglossus	B 4	. . . .		+	
"	F 23	. . . .		+	
"	C 27	. . . .		+	+
Rana fusca	M 3	. . . .		+	
"	U 4	. . . .	+		
"	U 13	. . . .	+		
Rana esculenta	E 1	. . . .	+	+ +	
"	G 2	. . . .			+
"	G 7	. . . .		+	+
"	G 8	. . . .		+	
"	G 10	. . . .			+
"	G 12	. . . .	+	+	
"	G 13	. . . .	+		
"	G 18	. . . .			+
"	G 20	. . . .	+		'
"	G 22	. . . .	+		
"	G 24	. . . .	+		
"	G 25	. . . .	+		
"	H 1	. . . .	+	+	
"	H 3	. . . .			+
"	J 9	. . . .	+	+	

It is immediately obvious that the blastoporal lip does not always *1658* appear on the animal side, as would have had to happen if the dorsal factor had been pushed back by the vitellus or linked to it in some manner. In return, these results agree excellently with the hypothesis of a rigid cortical field. The blastoporal lips actually appear indifferently on the animal end (10 cases), vegetative end (11 cases), or lateral side (6 cases); the term "indifferently" is used here since, in one and the same embryo, two blastoporal lips are able to form (an. + veg.: R. esc. E 1, G 12, H 1, J 9; veg. + lat.: Disc. C 27 and R. esc. G 7). The proportion of double embryos is relatively high (6/21) whereas, in rotations through $135°$, the vitelline mass which only changes place loses little of its extent. In this respect, we should mention that, if the vitelline mass is displaced in the opposite direction away from the dorsal region, there will never be double embryos. We will return later to these rotations through $135°$, which will be analyzed in detail in a future paper. For the moment, let us recall that the hypothesis of a rigid dorsoventral cortical field is the only hypothesis agreeing with the totality of the experimental results.

c) How should the combination of the vitelline gradient and the cortical field be understood? Do the two factors play an equal role or is one of them subordinate to the other? One would be tempted to attribute the major role to the dorsoventral factor. Would the effect of the vitellus not be entirely mechanical? Should the cortical plasm, which constitutes the premorphogenic substances, have to await a certain cytogenesis to become capable of inducing gastrulation and would this not be favored by the purely mechanical contact of a vitelline mass? With respect to such cellularization, let us mention that, although it is well possible that a certain cell formation is necessary to have

gastrulation take place, this condition is by no means sufficient. Let us take the typical example of Figs.2 and 3 (pp.10-11). It is obvious that, all around the vitelline conglomerate in the entire marginal zone, all cells having the same vitellus content should have approximately the same size. However, <u>/659</u> gastrulation appears first at the dorsal end and, after a delay of several hours, at the ventral side.

With respect to the contact of the vitelline mass, this factor is secondary in nature. There is no doubt that an organizer, transplanted to the animal pole far from the vitelline mass, will undergo there a difficult and highly aberrant evolution, but its activity will remain considerable. However, the region corresponding to the organizer which, starting from the stage VIII, is separated from the vegetative portion of the ovum (experiment on isolation of micromeres by Vintemberger, 1935-36), will show no activity at all. Thus, it is certain that the factors which caused the morphogenesis must have undergone - during the entire interval of segmentation and blastulation - a profound modification. It is not too venturesome to assume that this modification must be of a chemical type, obeying the law of mass action. In fact, at equal vitelline concentration, gastrulation will always appear first at the point at which the cortical field has its greatest density. Conversely, if the conditions of the field are identical, or more or less so, the activity will be greatest at the base of the vitelline gradient. It should be mentioned in this respect that the gastrulation appears first at the base of the vitelline gradient and that increasingly less vitelline territories then invaginate successively; it is in this manner that the cephalocaudal axis would be established, determined directly by the vitelline gradient. Similarly, the cephalic organizer, which contains the organizatory substances in a greater quantity than the truncal organizer (see Dalcq, 1936), will also form at the base of the vitelline gradient. Considering now the dorsoventral progression of the blastoporal lip, it will be found that the order of appearance of the dorsal, lateral, and ventral lips runs distinctly parallel to the decrease of the field.

In our joint paper, Dalcq and myself (1937) symbolized this evolution by the reaction C × V which constitutes the product CV. We stipulated that definite thresholds of these products are necessary for the initiation of invagination, induction, etc. Since the reaction obeys the law of mass action, it is <u>/660</u> easy to understand that these thresholds will be reached at sites where C (cortical field) and V (vitellus) are most abundant. Thus, we assumed - in first analysis - that the vitellus participates in reactions causing the prodromes of morphogenesis. This obviously represents only a first approximation since we do not know whether a disintegration product of the vitellus must necessarily combine with the hypothetical substance of the field to induce the start of the premorphogenic metabolism. It might also be possible that the latter is quantitatively influenced by a physicochemical condition of the vitellus which acts as a simple vehicle for this condition. In this respect, let us mention that Dorfmann and Grodsensky (1937) recently showed, based on methods that seem more convincing than those of their predecessors, that the vegetative pole of the frog ovum is distinctly more acid than the animal pole. For lack of more information, future data must be awaited. However, no matter how this might be, whether the vitellus actively participates in the reaction or intervenes only by the effect of its mere presence, the result is the same: The premorphogenetic metabolism remains proportional to the quantity of vitellus. Thus, in first

analysis, the symbol C × V is in any case fully applicable.

Thus, it is now possible to understand the formation of double embryos in the following manner, referring to Fig.8: The diagram gives a schematic view over the possibilities analyzed above: a) A large vitelline conglomerate exists at the level of the vegetative pole (Fig.8a). The vitelline decrement radiates around this large plaque in all directions (broken lines); the focus of the cortical field is marked by an asterisk at the top of the diagram. Among all boundary points that outline the vitelline mass, it is easy to see that the point a, closest to the focus of the field, will be more predisposed than all others to form a dorsal lip of the blastopore. The morphogenesis originating at this single point will necessarily be unitary; in fact, the morphogenetic /661 system, having suffered its initiation at the point a, will progressively exert its influence on the entire vitelline margin, i.e., on the points b and c and finally on the point d, before these points — too far away from the focus of the field — will have had the time to start, in turn, an initiation of morphogenesis. b) Only a small vitelline conglomerate exists at the level of the vegetative pole (Fig.8b). A first blastoporal lip forms at the point a. The point d, opposed to the latter, is quite close to it. The intensity of the field between a and d is thus rather similar. The embryonic system, starting at the point a, may exert its influence up to b and c without, however, reaching the point d at the instant at which this latter is able, in turn, to constitute a dorsal lip of the blastopore. c) A vitelline streak has formed along a segmentation groove, which is assumed to pass through the center of the gray crescent in Fig.8c but which, in other cases, may be lateral and thus pass at some distance from this center. On either side of this streak, two vitelline gradients radiating in opposite directions are present; these are separated only by a highly reduced or even nonexistent zone of white cells. Along the entire streak, a series of points a and b, c and d, etc. exist which are isopotential in their vitelline properties as well as in their position relative to the field. At the point closest to the center of the gray crescent, i.e., at the level of the focus itself in the example selected by us, two osculating blastoporal lips will form at the base of the two vitelline gradients.

The three possibilities schematically shown above reproduce the essential aspects of the described cases. Rana esculenta A 16 is an example for the type a; Rana fusca C 13 and E 16 illustrate the evolution of the type b; Rana fusca D 1 belongs to the category c. The presence of a supplementary vitelline conglomerate at the animal pole partly explains the particular evolution of R. f. K 2 and of R. esc. D 4; for these same embryos as well as for R. esc. Fl, the influence of the "gray crescent" factor manifests itself especially in the form of expansions and deformations of the blastoporal lip in direction of the /662 central point of this crescent.

The outlined interpretation, in addition to what has transpired so far, presupposes another condition: The nascent blastoporal lip must be conceived as the central point of a veritable embryonic system whose influence is felt at some distance from this point. In fact, if a small vitelline conglomerate exists at the surface of the ovum, the latter will surround itself with two distinct blastoporal lips; it is characteristic here that these blastopore lips are always diametrically opposed. In the case schematically shown in Fig.8b (see R. f. C 3 and E 6), a first lip is formed facing the gray crescent at the

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level of the point a; the second lip is formed later at the point d which is the most ventral point. This can be understood only if the entire dorsal half of the marginal zone, extending from a to b and c, is literally "neutralized" by the action of the system, namely the morphogenetic field whose blastoporal lip, forming at the point a, constitutes the true focus. The propagation of the morphogenetic wave along a vitelline conglomerate (or of the virtual blastopore of normal development) on the whole is nothing else but a corollary of this progressive neutralization. It will be shown below (rotations through 135°) that a blastopore lip, which just about is becoming outlined, can be integrated into a more powerful neighboring morphogenetic system. In this respect, a comparison suggests itself: The interpretation given recently by Luther (1936-1937) of his highly interesting experiments made on the trout ovum agrees remarkably well with the concept outlined here. Arbitrary blastula fragments (Luther, 1936), cultivated in the umbilical vesicle (vitellus contact) of an older embryo indistinctly form notochord, mesoblast, and nervous system. At the instant at which the first blastoporal lip appears, these differentiations become restricted to the dorsal portion of the blastodisk whereas the ventral zones will have lost these potentialities; thus, it must be assumed that, also in the Teleosteae, the dorsal blastoporal lip, from the instant of its appearance, constitutes the $\underline{/663}$ central part of a system and exerts a true physiological dominance over the peripheral territories. The same conclusion can be drawn from the 1937 experiments during which Luther, among others, made transplantations of extra-embryonic sectors (varying from 45° to 90°) at the center of the organizer zone. At the level of the two contact zones of the implanted sector with the host, an embryonic axis may form; the lateral portions of the organizer actually remain in place (see Luther, 1935) and may be adequate for inducing an embryonic axis at the level of the lateral portions of the inactive transplant. Luther was entirely justified to state that, allowing for the usual risk of the operation which is rather delicate to perform, three possibilities can be expected: a) formation of two equal embryonic axes on either side of the transplant; b) formation of two unequal axes; c) formation of a single embryonic axis. However, in the totality of the results reported by the author, only the possibilities a) and c) are mentioned. The explanation given by Luther himself is as follows: In the first case (a), the two fragments of the organizer exert an attractive effect on the less active neighboring regions, where the two actions balance out since the two fragments are equal; in the second case (b), one of the fragments which is preponderant entrains the weaker fragment into its orbit. Consequently, the case b) is automatically reduced to the possibility c).

Let us mention, however, as remarked by Dalcq himself at the last session of the Association of Anatomists (meeting at Basle; see also Dalcq and Pasteels, 1938), that the physiological gradient and the physiological dominance, although they are to be understood completely in the sense given to them by Child, by no means constitute initiating factors. They merely represent mechanisms which themselves are derivatives of a pre-established structure, namely, the primordial organization of the nonfertilized ovum (vitelline gradient – cortical field – genoneme).

No matter how this might be, Luther believed himself authorized to state, ever since 1936, that "the dependence of organ differentiation on an 'organizer' does not exist from the very beginning <u>as is the case for Amphibia</u>[*] but that it

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[*] Underlining by us.

had formed gradually during the course of evolution, in the sense of a gradient that can be considered a 'physiological gradient field' (Child) which spreads /664 from the major axis of the germ toward both sides in the form of a ring". This is exactly what we had emphasized. Under the reservations made above, i.e., that the factors of the "physiological gradient" will have to be defined, Luther's conclusion can be applied word for word to amphibians; the differentiation from amphibians, made by the author himself, is entirely artificial.

We have demonstrated above (see Introduction) that the assumption of a pre-existence of the organizer in Amphibia from the youngest stages is not justified. Thus, it is completely paradoxical to see Penners and Schleip, having available all elements for proving the absence of preformation of the organizer, nevertheless insist on this preformation. We have seen the confusion resulting from this attitude. Nevertheless, the situation seems entirely clear: In the case of Fig.9b, it is evident that the two blastopores, namely the dorsal and the ventral, form in virtue of the same mechanism and that only quantitative differences explain their chronological divergence; it is also obvious that the single dorsal blastopore in Fig.9a will form in virtue of these same conditions. In addition, it is evident that the blastopore of a normal ovum will form under the effect of exactly the same factors. However, what is valid for the dorsal lip of the blastopore and for the organizer must thus be just as valid for all prodromes of morphogenesis. In fact, it is quite certain that in a "morpho-genetic field" manifesting itself in the appearance of an embryonic axis in a ventral territory of the ovum, creation of the organizer, tendency to morpho-genetic movements, normal distribution of presumptive anlage, and local pre-dispositions must have taken place in territories of the ovum where, in the /665 normal course of development, these elements would not have existed. This means that, in the normal development, these prodromes of morphogenesis, constituting a true constellation coordinated around the blastoporal focus, are also created entirely at the expense of a much more elementary structure. Segmentation and blastulation, far from being "neutral" from the morphogenetic viewpoint, actual-ly constitute one of the (most important) links of a continuous chain of events.

An analysis, from the biological as well as from the physicochemical view-point, of this particular stage which until now had been totally neglected re-mains one of the most urgent problems of present-day embryology.

4. Summary

The inversion of ova before segmentation (or at the very beginning of seg-mentation) of Rana fusca, Rana esculenta, and Discoglossus pictus led to the following conclusions.

a) Results

1) A blastoporal lip just as all prodromes of morphogenesis (organizer, morphogenetic movements, local predispositions) are able to form at all points of the ovular surface.

2) The two conditions necessary for the appearance of these prodromes are:

contact of large vitelline masses with the ovular cortex, and a factor having its intensity maximum on the dorsal end.

3) The vitelline gradient determines the cephalocaudal polarity.

b) Interpretation

1) The prodromes of morphogenesis would result from an interaction of a chemical nature between the vitelline gradient and a cortical substance distributed over a field whose intensity decreases progressively from a dorsal /666 focus: the dorsoventral cortical field.

2) The dorsal lip of the blastopore would be the true nodal point of a complex system and would exert a dominant physiological action on the less developed lateroventral regions.

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